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Compromise of the hypoglycaemic effect of chlorpropamide by the co-administration of *Vernonia amygdalina* (Compositae) aqueous extract

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Abstract
Most diabetes mellitus patients practice self-administration of herbal products along with their conventional drugs without the knowledge of their physicians. *Vernonia amygdalina* is widely used for its therapeutic and nutritional purposes have been reported to have appreciable hyperglycaemic activity. This study evaluated the biological implication of co-administration of *V. amygdalina* (aqueous extract) with chlorpropamide an oral hypoglycaemic agents using alloxan induced animal model. Body weight (BW) and blood glucose level (BGL) of the animals was monitored during the study while haematological, biochemical and histopathological evaluations were determined at the end of the study using standard methods. Significant increase in BW and reduction in BGL was observed in all the treated groups when compared with diabetic control by day 21 ($p<0.05$), although the rate of blood glucose was slow with the co-administration. Reduction in the mean cell volume (MCV), mean cell haemoglobin (MCH), serum total protein and albumin ($p= 0.024$), while serum urea, total cholesterol and creatinine levels were increased in the co-administration when compared with the extract and chlorpropamide alone. Severe wide spread of vacuolar degeneration and moderate polymorphonuclear cell degeneration of the liver and vacuolar congestion of the cortex medulla of the kidney was observed with the co-administration. The outcome of this study revealed that co-administration of *V. amygdalina* and chlorpropamide does not have any overall beneficial effect as it subject to compromise of the hypoglycaemic effect as well as possible complication of the disease condition.

Keywords: *V. amygdalina*, Chlorpropamide, Co-Administration, Hypoglycaemic effect; Compositae

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder of the endocrine system resulting from insulin deficiency or insulin dysfunction. It can be classified as Type 1 (insulin dependent diabetes mellitus, IDDM), Type 2 (non-insulin dependent diabetes mellitus, NIDDM) and gestational diabetes (GDM. Type 2 diabetes which is as a result of insulin dysfunction constitutes almost 90% of the diabetic population [1] is managed by the use of diet, exercises and medication [2]. Diabetes is a multi-factorial disease which is characterized by hyperglycemia, hyperlipidemia, glycosuria and ketoacidosis, which results in severe complications [3,4]. It

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is usually associated with decreased quality of life and increased risk factor for morbidity and mortality.

The prevalence of diabetes has assumed an epidemic level with up to 8.5% of the population worldwide [5]. The current drug management of diabetes comprises the use of oral antidiabetic agents such as sulphonylureas (chlorpropamide, glibenclamide), α- glucosides (miglitol) and thiazolidinediones (rosigilatzone), biguanides (metformin) [6]. The chronic nature of diabetes as well as the side effects of most of these drugs is a cause of serious concerns to most patients, hence the interest in alternative mode of management of the disease [7, 8].

Folkloric use scientific reports on the biological activities of plants and plant products have encouraged interest in the use of such medicinal plants and their extract in the management of chronic diseases such as diabetes mellitus and hypertension.

Several species of medicinal plants have been reported to have hypoglycaemic activity, examples include, *Azadiracta indica* (neem tree) [9, 10], *Vernonia amygdalina* (Bitter leaf) [11, 12], *Ocimum gratissimum and Allium sativum* (garlic) [13, 14]. The perceived effectiveness, fewer side effects, relative low costs have encouraged the use of herbal remedies along with the prescribed orthodox drugs in other to reduce the cost of long term management of Type 2 diabetes [15, 16].

There has been an increased global use of herbal products as add-on therapy in disease management with most of the patients not disclosing the use of herbal preparations to their physicians. The concomitant use of herbal preparation with orthodox drugs has been reported across the different countries of the world [17, 18].

Previous workers have reported the use of a variety of herbs such as *Allium cepa, Allium sativum,* and *Zingiber officinale* along with conventional drugs by patients during the management of diabetes mellitus [19, 20]. Co-administration of herbs with orthodox drugs has been reported to be accompanied with a variety of biological actions as a result of pharmacodynamic and pharmacokinetic interactions [21]. Some of these interactions may result in potentiating the pharmacological effect of the drug and or severe adverse reactions [18, 22, 23].

Allium sativum [24], *Aloe vera* [25], *Gymnema sylvestre* [18] and *Vaccinium myrtillus* (Bilberry leaves) [26] have been reported to lower blood sugar thus enhancing hypoglycaemia. Garlic was reported to severely enhance hypoglycaemia when administered with metformin [27]. Consumption of crude prickly pear cactus with glipizide and metformin was reported to cause severe hypoglycaemia as a result of additive effect [28].

Vernonia amygdalina (family Compositae) commonly known as bitter leaf is used for medicinal and nutritive purposes in Nigeria, as well as the African sub-region. The leaves are used medicinally for a variety of conditions ranging from bitter stomachic, malaria, diarrhoea and diabetes [29, 30]. The hypoglycaemic activity of the leaves on alloxan-induced diabetes has been reported by different workers [31, 32, 33].

The reported prevalence of the concomitant use of *Vernonia amygdalina* with antidiabetic agents such as chlorpropamide [34] necessitates the need to evaluate the biological implications of such co-administration in view of the scarcity of such reports in our environment. Hence, this study was aimed at evaluating clinical implications of co-administration of the aqueous leaves extract of *Vernonia amygdalina* with chlorpropamide, a known antidiabetic drug.

EXPERIMENTAL

Plant material. Fresh leaves of *Vernonia amygdalina* (family Compositae) were

collected from the Botanical Garden of the University of Ibadan. It was identified and authenticated at the Forest Research Institute (FRIN) Ibadan, Nigeria with voucher specimen deposited in the herbarium with number FHI/109843.

Phytochemical screening. The plant was subjected to phytochemical screening at the Department of Pharmacognosy, University of Ibadan [35].

Preparation of aqueous extract. The fresh leaves were washed with distilled water without squeezing, air dried and pulverized into powder. The dried powdered leaves (100g) was extracted by cold maceration method using 1000ml of distilled water with intermittent shaking for about four hours, and allowed to stand for 72 hours before filtering. The resultant filtrate was evaporated to dryness using a freeze dryer; the obtained residue was stored in an air tight container at 4^oC.

Identification and assay of chlorpropamide. Chlorpropamide (2^o Standard) was identified by melting point determination, thin layer chromatography, while the chemical content was determined using the official method [36].

Herb-drug interaction study

Experimental animals. Twenty-five healthy male Wister rats (150-220g) obtained from Central Animal House, College of Medicine, University of Ibadan were used for the study. The rats were allowed to acclimatize over a period of one week, maintained under standard laboratory conditions [Temperature: 25 – 30^oC, 12-hour light and 12-hour darkness cycles] during which they were fed with rat pellets (Ladokun feeds, Ibadan, Nigeria) and water was given *ad libitum*. The rats were randomly distributed into five groups (n=5) based on the research design. The animal study was done in accordance with the National Institute of Health Guidelines for Care of Laboratory animals of 1985.

Research design. The following research groups were used for the study;

- *HG – Negative control, administered DMSO in water (10%w/v)*
- *DNC - Diabetic positive control, induced with diabetes administered DMSO in water (10%w/v).*
- *DVA – Diabetes induced administered V. Amygdalina aqueous extract (100mg/Kg)*
- *DC - Diabetes induced administered Chlorpropamide (5mg/Kg)*
- *DVC-Diabetes induced administered V. Amygdalina aqueous extract (100mg/Kg) and Chlorpropamide (5mg/Kg)*

Induction of diabetes. After an overnight fast, twenty rats were induced with hypoglycaemia using alloxan monohydrate solution (100mg/kg) (Sigma, USA) intraperitonially. Diabetes was confirmed in all the induced rats with random blood glucose level ≥ 200 mg/dl after 72 hours using a digital glucometer (Accu-Check®, Roche, India).

Dose administration. Solutions of chlorpropamide (0.18% w/v) in DMSO (10%v/v) and *V. Amygdalina* (9%w/v) in distilled water were prepared and administered to the rats based on their body weights and the research design at 100mg/kg for the aqueous *V. Amygdalina* [32], and 5mg/Kg for chlorpropamide. The administrations were done orally using oral cannula once daily for 21 days. The animals were regularly observed for their general behaviour for the 21 days.

Determination of weights and glucose levels. The body weights of the animals were determined using a digital top loader weighing balance before the commencement of drug administration (day 0) and on days 1, 3 7, 14, 21 of the study. Blood glucose level was determined using a digital glucometer (Accu-Check®, Roche, India) from the tail snips on days 0, 1, 3, 7, 14 and 21 days.

Determination of haematological parameters. At the end of the study; 24hours after the last administration blood samples (5ml) were collected from the rat through the retro-orbital puncture into heparinised bottles. The collected blood was use to determine packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood count (WBC), differential white blood cell count (neutrophil, lymphocytes, monocytes and eosinophils), erythrocyte sedimentation rate (ESR) and platelet count. Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were obtained by calculation [37].

$$MCV = \frac{PCV \times 100}{RBC\ count\ (10^6/mm^3)}$$

$$MCH = \frac{Hb\ (g/dl) \times 100}{RBC\ (10^6/mm^3)}$$

$$MCHC = \frac{Hb\ (g/dl) \times 100}{PCV\%}$$

Evaluation of biochemical parameters. Another set of blood (5ml) was collected into labelled non-heparinised bottles, allowed to clot in a slanting position before centrifuging at 3000r.p.m for 10 minutes. The serum obtained was transferred into another set of clean labelled sample bottles and stored in a freezer at -4^{o}C until it was analysed. The obtained serum was used to determine total protein, albumin, urea, creatinine, total cholesterol, liver enzymes; alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using Randox test kits (Randox Laboratories).

Histopathological evaluation. The rats were euthanized by cervical dislocation at end of the study, and the kidney and liver were harvested. Histopathological examination using conventional methods; stained with haematoxylin and eosin (H/E) was carried out at Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan.

Statistical analysis. Data were expressed as mean ±S.E.M. and subjected to statistical analysis using Student t-test and ANOVA with the Duncan's Multiple Range test used for the post test where appropriate. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The quality and chemical content of the chlorpropamide (2^o standard) complied with the official specification based on the melting point (126^{o}C-128^{o}C), TLC showing single spot (R_f = 0.78) and chemical content of 100.1%w/v [36]. This study confirmed the presence of alkaloids, tannins, saponins, cardenolides, anthraquinones and flavonoids in the *Vernonia amygdalina* leaves which are in line with previous reports [32]. Flavonoids are reported to exhibit antioxidant activity and are effective scavengers of superoxide anions [38]. The presence of flavonoids in *V. amygdalina* extract has been associated with stimulation of insulin secretion by enhancing hepatic glucokinase activity [39, 40].

The yield of the aqueous *V. amygdalina* extract obtained was 8.8%w/w with the TLC analysis showing the presence of alkaloids among the identified spots based on the visualization method.

Investigation into chlorpropamide, *V. Amygdalina* aqueous extract and their co-administration caused significant decrease in blood glucose level which was accompanied by significant increase in weights ($p < 0.05$) (Figures 1 and 2).

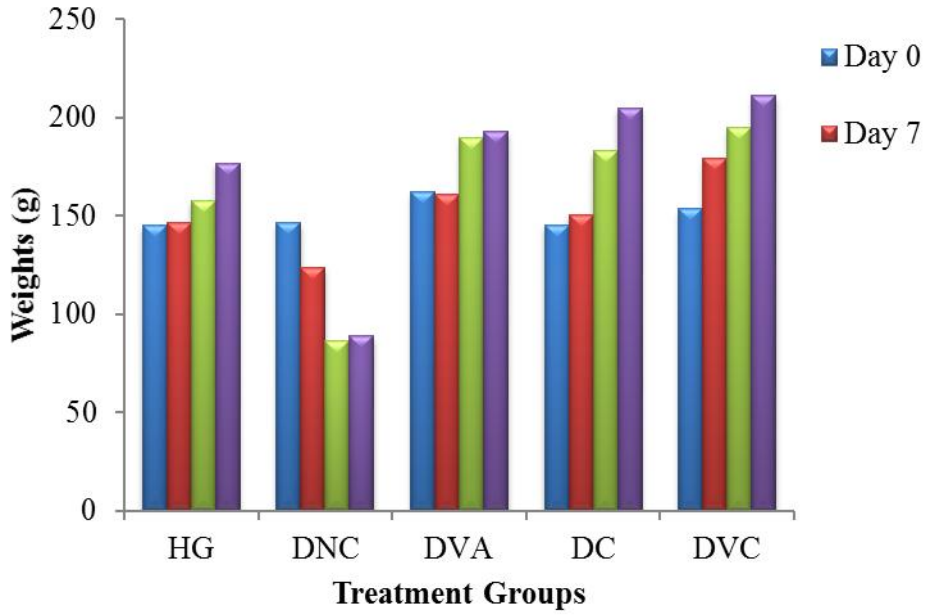


Figure 1: Effect of administration of chlorpropamide (DC), *V. amygdalina* aqueous extract (DVA) and their co-administration (DVC) on the body weights of male Wistar rats. [Code: Healthy rats (HG), Diabetic Control (DNC)]

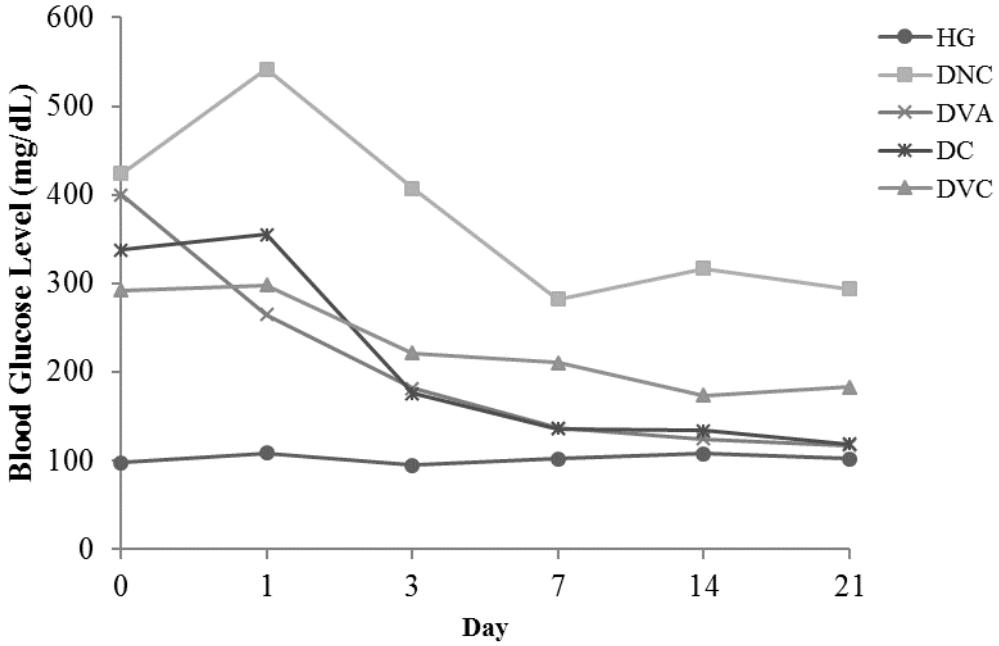
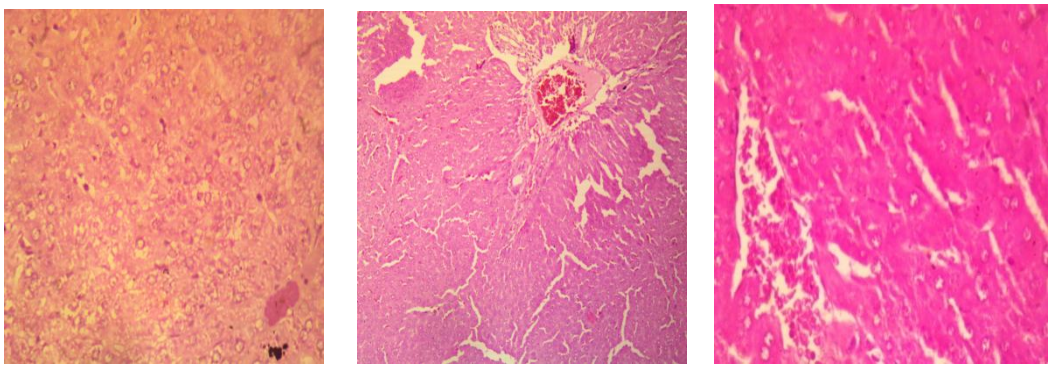
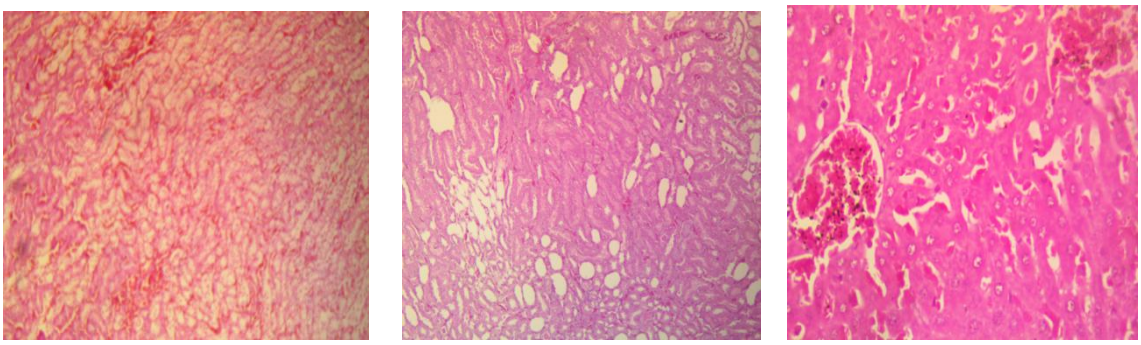


Figure 2: Effect of administration of chlorpropamide (DC), *V. amygdalina* aqueous extract (DVA) and their co-administration (DVC) on the Blood Glucose Level (BGL) of male Wistar rats. [Code: Healthy rats (HG), Diabetic Control (DNC)]



DVC DC DVA

Figure 3: Photomicrograph of the liver of diabetic rat treated with chlorpropamide (DC), *V. amygdalina* (DVA) and their co-administration (DVC) [DVC (x100), DC and DVA (x40)]



DVC DC DVA

Figure 4: Photomicrograph of the kidney of diabetic rat treated with chlorpropamide (DC), *V. amygdalina* (DVA) and their co-administration (DVC) [DVC (x100), DC and DVA (x40)]

Table 1: Effect of administration of chlorpropamide, V. amygdalina aqueous extract and their co-administration on the haematological parameters after 21 days to male Wistar rats

Treatment Code	Haematological parameters									
	PCV (%)	Hb (g/dL)	RBC ($x10^{12}$/L)	WBC ($x10^{9}$/L)	Platelet ($x10^{9}$/L)	Lympho cytes (%)	Neutro phils (%)	MCV (fl)	MCH (pg)	ESR
HG	51.8 ±0.4	17.2 ±0.2	5.8 ±0.9	9.7 ±1.9	15.0 ±0.0	60.4 ±0.2	39.6 ±0.2	103.6 ±23.9	34.0 ±8.0	4.8 ±0.2
DNC	42.3 ±4.9	14.0 ±1.6	8.3 ±2.5	8.8 ±0.2	12.3 ±1.5	75.0 ±1.5	35.0 ±1.5	33.0 ±0.0	10.0 ±1.0	7.3 ±1.3
DVA	47.0 ±0.6	15.4 ±0.2	6.7 ±1.4	11.1 ±1.3	14.0 ±0.0	61.0 ±5.5	42.3 ±2.2	77.0 ±19.3	24.7 ±5.8	2.0 ±0.0
DC	47.8 ±0.3	15.9 ±0.5	7.9 ±1.9	10.5 ±0.7	14.0 ±0.0	65.5 ±0.3	34.5 ±0.3	71.3 ±17.6	23.2 ±5.7	3.8 ±0.3
DVC	49.3 ±0.3	16.4 ±0.2	12.4 ±0.1	7.7 ±1.5	14.0 ±0.0	70.3 ±0.3	29.7 ±0.3	39.3 ±0.3	13.0 ±0.0	1.7 ±0.3

HG = Healthy rats (negative control); DNC = Diabetic Normal Control (without treatment); DVA = Diabetic + *V. amygdalina*; DC = Diabetic + Chlorpropamide; DVC = Diabetic + Chlorpropamide + *V. amygdalina*

Table 2: Effect of administration of chlorpropamide, V. amygdalina aqueous extract and their co-administration on the biochemical indices after 21 days to male Wistar rats

Treatment Code	Biochemical indices (mg/ dL)					Liver Enzymes (mg/ dL)	
	Total Protein	Albumin	Urea	Creatinine	Total cholesterol	ALT	AST
HG	7.37 ±0.17	4.52 ±0.20	39.33 ±3.47	0.28 ±0.09	168.00 ±8.89	23.60 ±1.92	29.48 ±6.85
DNC	5.66 ±0.48	2.87 ±0.61	100.07 ±0.13	1.97 ±0.29	178.00 ±9.00	44.36 ±0.57	104.17 ±5.77
DVA	6.72 ±0.21	4.62 ±0.17	40.35 ±6.12	0.28 ±0.13	141.00 ±13.00	26.25 ±0.29	84.04 ±9.42
DC	7.13 ±0.32	3.73 ±0.52	44.24 ±2.76	0.38 ±0.24	169.25 ±4.91	25.99 ±4.63	64.48 ±4.66
DVC	6.47 ±0.57	3.16 ±0.37	45.37 ±5.91	0.66 ±0.20	176.50 ±11.50	35.11 ±1.33	90.35 ±5.09

HG = Healthy rats (negative control); DNC = Diabetic Normal Control (without treatment); DVA = Diabetic + *V. amygdalina*; DC = Diabetic + Chlorpropamide; DVC = Diabetic + Chlorpropamide + *V. amygdalina*

The extent of the increase in weights was more significant for DVA and DVC than for DC this shows that the aqueous extract enhances the increase in weight by chlorpropamide or vice-versa. The comparable hypoglycaemic activity of *V. amygdalina* extract with chlorpropamide was confirmed in this study which corroborates previous reports [29,31,33]. However, the reduction observed with co-administration was not as much as when the chlorpropamide and *V. Amygdalina* were used alone. This shows that the concomitant administration of *V. amygdalina* with chlorpropamide could reduce the blood glucose lowering effectiveness of both *V. amygdalina* and chlorpropamide when used alone. This indicates that much as there was a glucose lowering effect by the co-administration, the effect was antagonistic to the effect of chlorpropamide and the aqueous extracts of *V. Amygdalina* alone.

The effect of co-administration body weight indicates that the aqueous extract enhances the effect of chlorpropamide which shows a beneficial effect as the loss in body weight which is a feature of diabetes mellitus will be taken care of by the co-administration of the aqueous extract and chlorpropamide, but this may not be beneficial in obese patients. However, the effectiveness of the blood glucose lowering effect will be compromised.

All the haematological parameters were affected by the co-administration of *V. amygdalina* and chlorpropamide, although the effect was more significant on RBC and WBC with increase in RBC and reduction in WBC. Significant increase in lymphocytes and reduction in neutrophils, MCV, MCH and ESR levels (Table 1) by the co-administration compared to healthy state could be a source for concern as it could indicate a compromise of the immune system and clotting activity [41, 42].

The significant reduction in the serum total protein (0.0297) and albumin (p=0.024) by the herb-drug combination compared to the healthy state, *V. amygdalina* and chlorpropamide (Table 2) could be due to possible damage to liver and or increased intestinal protein loss [43]. Similarly, serum urea and creatinine levels were higher in the herb-drug combinations compared to the healthy group but were significantly reduced when compared with the diseased state ($p< 0.05$) which corroborates the possible beneficial hypoglycaemic effect. The increase in serum urea and creatinine levels by the diabetic state (Table 2) may be attributed to

failure of the body to excrete the metabolic end products of proteins which could not be reversed by the co-administration [44].

The decrease in serum protein and albumin, and increase in urea, creatinine and total cholesterol observed with co-administration of herb and drug were not significant when compared to chlorpropamide alone ($p > 0.05$). However, chlorpropamide significantly reduced the effect of the *V. amygdalina* on serum albumin ($p=0.0231$)

The serum total cholesterol reduced by the aqueous extract and chlorpropamide compared to the diseased state, though higher with the aqueous extract was not significant ($p>0.05$), however, the herb-drug co-administration had no such effect. The reduced total cholesterol level observed with V. amygdalina is in agreement with previous report by Akah *et al.* in 2009 [45]. This indicates that chlorpropamide significantly increase total cholesterol when co-administered with the aqueous extract of V. amygdalina (Table 2).

Diabetes mellitus is usually accompanied by altered lipoprotein metabolism resulting in macrovascular complications as observed in increased serum total cholesterol in untreated diabetic rats which is in agreement with earlier reports [46, 47]. The result of this study showed though chlorpropamide and *V. amygdalina* alone could alleviate this condition; their co-administration may not be able to alleviate the macrovascular complications.

Transaminases (aspartate transaminases, AST and alanine transaminases, ALT) are a well-known biomarkers used to predict possible hepatotoxicity [48]. Increase in the ALT and AST activities observed in the diabetic control is in agreement with earlier reports [49, 50]. Reduction in the enzyme activities by the herb-drug combination was not significant ($p>0.05$) when compared with diseased state, although significant reduction in AST was obtained with chlorpropamide ($p=0.0023$) when used alone, which was not significant with aqueous extract of *V. amygdalina* alone.

The significant reduction in the ALT agrees with the reported hepatoprotective effect of *V. amygdalina* [50, 51]. It is of note that none of the treatment groups could reduce the level AST activity to the healthy state, which was achieved with ALT by chlorpropamide (DC) and the extract (DVA) alone. This study thus shows that concomitant use of the aqueous extract with chlorpropamide is predisposed to hepatic problems.

This hepatotoxic effect was confirmed by the histopathological evaluation which revealed that the co-administration of *V. amygdalina* with chlorpropamide caused damage on both the liver and kidneys. The histopathological findings in the liver and kidneys are in agreement with the biochemical findings such as increased levels of serum creatinine, urea, ALT and AST due to cellular damage and increased membrane permeability of both the liver and the kidney [52]. In addition, untreated diabetic rats showed moderate vacuolation, loss of renal tubules with moderate infiltration of inflammatory cells which explains the increased creatinine and urea levels in these rats. In diabetes mellitus vacuolation of hepatocytes could arise from accumulation of glycogen in these cells – a condition referred to as hepatic glycogenosis of diabetes mellitus (glycogenic hepatopathy) [50].

The assessment of some kidney function indicators; urea and creatinine levels shows amelioration/ modulation of the potential risk posed by diabetes to the kidneys by the aqueous extract such as decreased urea and creatinine levels, which was increased in the untreated diabetic group. This effect is similar to kidney protective action of *V amygdalina* extract earlier reported [50] which is confirmed by the normal structure

obtained for the kidneys in the extract group (DVA) (Figure 3).

On the hand, the severe congestion and vacuolar degeneration of medulla cortex with severe polymorphonuclear cell infiltration by *V amygdalina* and mild diffused capillary congestion in the cortex of the medulla seen with chlorpropamide is confirmed by the elevated urea and creatinine levels (Table 2, Figure 3).

Similarly, this study reported a normal structure for the liver by the extract which confirms the low ALT and AST activity levels, while the severe congestion and vacuolar degeneration of medulla cortex obtained with herb-drug co-administration was corroborated by the elevated ALT and AST levels (Table 2, Figure 4).

Evidence from this investigation indicates that co-administration of aqueous *V amygdalina* extract with chlorpropamide is accompanied by various levels of risks; antagonistic effect on the hypoglycaemic effect of the extract and chlorpropamide as well as possible complications of the diabetic conditions as a result of the deleterious effects on the key organs involved the physiology diabetic condition.

Previous reports on the implication of herb-drug co-administrations shows variation in outcome ranging from adverse effects, antagonism, additive and synergy as a result of differences in mechanism of action and pathogenesis of disease conditions. This indicates that until each herb-drug co-administration is verified there should be caution in such practice. Unfortunately, most patients do not disclose their involvement in this practice to their physicians, which further complicates the issue.

Conclusion. The outcome of this investigation showed that co-administration of *V amygdalina* and chlorpropamide does not have any overall beneficial effect as it subject to compromise of the hypoglycaemic effect as well as possible complication of the disease condition. Hence, there is the need for appropriate counselling of patients by health care providers on the possible risks and benefits of specific herbal medicines, just as they do with conventional medicine.

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